

Figure 1: Human POSH Coding Sequence (SEQ ID NO:1) (part 1)

ATGGATGAATCAGCCTTGTTGGATCTTTTGGAGTGTCCGGTGTGTCTAGAGCGCCTTGATGCTTCTGCGA
AGGTCTTGCCCTTGCCAGCATACGTTTTGCAAGCGATGTTTGCTGGGGATCGTAGGTTCTCGAAATGAACT
CAGATGTCCCAGTGCAGGACTCTTGTGGCTCGGGTGTGAGGAGCTTCCCAGTAACATCTTGCTGGTC
AGACTTCTGGATGGCATCAAACAGAGGCCCTTGGAAACCTGGTCTGGTGGGGGAAGTGGGACCAACTGCA
CAATGCATTAAAGGTCTCAGAGCAGCACTGTGGCTAATTGTAGCTCAAAAGATCTGCAGAGCTCCCAGGG
CGGACAGCAGCCTCGGGTGCATCCTGGAGCCCCCAGTGAGGGGTATACCTCAGTTACCATGGCCAAA
GCGTTATACAACCTATGAAGGAAAAGAGCCTGGAGACCTTAAATTAGCAAGGCGACATCATATTTTGC
GAAGACAAGTGGATGAAAAATTGGTACCATGGGGAAGTCAATGGAATCCATGGCTTTTTTCCCACCAACTT
TGTGCAGATTATTAACCGTTACCTCAGCCCCCACCCTCAGTGCAAAGCACTTTATGACTTTGAAGTGAAA
GACAAGGAAGCAGACAAAGATTGCCTTCCATTTGCAAGGATGATGTTCTGACTGTGATCCGAAGAGTGG
ATGAAAACCTGGGCTGAAGGAATGCTGGCAGACAAAATAGGAATATTTCCAATTTTATATGTTGAGTTTAA
CTCGGCTGCTAAGCAGCTGATAGAATGGGATAAGCCTCCTGTGCCAGGAGTTGATGCTGGAGAATGTTCC
TCGGCAGCAGCCAGAGCAGCACTGCCCCAAGCACTCCGACACCAAGAAGAACCAAAAAAGCGGCACT
CCTTCACTTCCCTCACTATGGCCAACAAGTCCTCCAGGCATCCAGAACCGCCACTCCATGGAGATCAG
CCCCCTGTCTCATCAGCTCCAGCAACCCCACTGCTGCTGCACGGATCAGCGAGCTGTCTGGGCTCTCC
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CTAAAGTCCCTATGTCTACAGCTGGCCAGACAAGTCGGGGAGTGACCATGGTCAGTCTTCCACGGCAGG
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CCACAACCTGCGCTCTGATGCCAGGCTCAGCCACGCACACTGCTGCCATCAGTATCAGTCGAGCCAGTG
CCCCTCTGGCCTGTGTCAGCAGCTGCTCCACTGACTTCCCCAAGCATCACCAAGTGTCTCTGGAGGCTGA
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CCTCCTCAGAGTGAGGCAGAACTTGAACCTAAAGAAGGAGATATTGTGTTTGTTCATAAAAAACGAGAGG
ATGGCTGGTTCAAAGGCACATTACAACGTAATGGGAAAACCTGGCCTTTTCCAGGAAGCTTTGTGAAAAA
CATATGA

Figure 2: Human POSH Amino Acid Sequence (SEQ ID NO:2) (part 2)

MDESALLDLLECPVCLERLDASAKVLPCHTFCKRCLLGIVGSRNELRCPECRTLVGSGVEELPSNILLV
RLLDGIKQRPWKPGPGGGSGTNCNLRSSSTVANCSSKDLQSSQGGQQPRVQSWSPFVRGIPQLPCAK
ALYNYEGKEPGDLKFSKGDIIILRRQVDENWYHGEVNGIHGFFPTNFVQIIKPLPQPPPQCKALYDFEVK
DKEADKDCLPFAKDDVLTVIRRVNENWAEGLADKIGIFPISYVEFNAAKQLIEWDKPPVPGVDAGECS
SAAQSSSTAPKHSSTKNTKKRHSFTSLTMANKSSQASQNRHSMELSPVLISSSNPTAAARISELSGLS
CSAPSQVHI STTGLIVTTPPPSSPVTTGPSFTFPSPDVYQAALGTLNPPPLPPPLLAATVLAATPPGATAA
AAAAGMGPRPMAGSTDQIAHLRPQTRPSVYVAIYPYTPRKEDELELRKGEMFLVFERCQDGFPGTSMHT
SKIGVFPNGYVAPVTRAVTNASQAKVPMSTAGQTSRGVTMVSPSTAGGPAQKLQNGVAGSPSVVPAVV
SAAHIQTSPOAKVLLHMTGQMTVNQARNVTRVAHNQERPTAAVTPIQVQNAAGLSPASVGLSHHSLAS
PQPAPLMPGSATHTAASISIRASAPLACAAAAPLTSPTSITSALEAEPSGRIVTVLPGLPTSPDSASSAC
GNSSATKPKDKSKKEKKGLLKLKLSGASTKRKPRVSPASPTELEVELGSAELPLQGAVGPELPPGGGHGRA
GSCFVDGDPVTTAVAGAALAQDAFHRKASSLDSAVPIAPPPRQACSSLGPVLNESRPVVCERHRVVVSY
PPQSEAELELKEGDIVFVHKKREDGWFKGTLQRNGKTGLFPGSFVENI

Figure 3: Human POSH cDNA Sequence (SEQ ID NO:3)

CTGAGAGACACTGCGAGCGGCGAGCGCGGTGGGGCCGCATCTGCATCAGCCGCCGAGCCGCTGCGGGG
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GGATGCACACAACATATGAACATTTCTGAAGATTTTTTCTCAGTAAAGTAGATAAAGATGGATGAATCAGC
CTTGTTGGATCTTTTGGAGTGTCCGGTGTGTCTAGAGCGCCTTGATGCTTCTGCGAAGGTCTTGCCTTGC
CAGCATACGTTTTGCAAGCGATGTTTGTCTGGGGATCGTAGGTTCTCGAAATGAACTCAGATGTCCCGAGT
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AACCGTTACCTCAGCCCCACCTCAGTGCAAAGCACTTTATGACTTTGAAGTGAAGACAAGGAAGCAGA
CAAAGATTGCTTCCATTTGCAAAGGATGATGTTCTGACTGTGATCCGAAGAGTGGATGAAAACCTGGGCT
GAAGGAATGTCTGGCAGACAAAATAGGAATATTTCCAATTTCAATGTTGAGTTTAACTCGGCTGCTAAGC
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TCTGATTTGCTATATATCCATACACTCTCGGAAAGAGGATGAACTAGAGCTGAGAAAAGGGGAGATGT
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AATGGGACCGAGGCCCATGGCAGGATCCACTGACCAAGATTGCACATTTACGGCCGCGAGCTCGCCCCAGT
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TGAGAGCAAGAGATTCGTTTTCATAGAGTGAATGCATTTTCCCTCTCCTCCTCCTGCTACCATAT
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TGGGTTTTTAAATTTTCTAGAATGAAGTGAAGTGAAGCAATGAGAAAGATAAGCACAACCTTGAAACA
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TTGAAGTTGTAGTCACTGTCTGAGAATGGCTATGAAGCGTCATTTACATTTTACCCCAACTGACCTGCA
TGCCAGGACACAAGTAAACATTTGTGAGATAGTGGTGGTGAAGTGAATGCACTCGTTAAGTCAAGGC
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GGATTCCCAAGTAAATAGACTGTGCATGGTGTGTATTTTCATTGCGATTTCTGTAAAGATGAGTTT
GTACTCAGAAATTGACCAATTGAGGAGGTGTAATAATAACAGTGTCTCTCTCTACCCCAAGCCACTA

-to be continued

9399577_1

Figure 3: Human POSH cDNA Sequence (SEQ ID NO:3)

CTGACCAAGGTCTCTTCAGTGCACTCGCTCCCTCTCTGGCTAAGGCATGCATTAGCCACTACACAAGTCA
TTAGTGAAAGTGGTCTTTTATGTCCTCCAGCAGACAGACATCAAGGATGAGTTAACCAGGAGACTACTC
CTGTGACTGTGGAGCTCTGGAAGGCTTGGTGGGAGTGAATTTGCCACACCTTACAATTGTGGCAGGATC
CAGAAGAGCCTGTCTTTTATATCCATTCCCTGATGTCTATGGCCTCTCCACCGATTTTCATTACGGTGC
CACGCAGTCATGGATCTGGGTAGTCCGGAAAAACAAAGGAGGGAAGACAGCCTGGTAATGAATAAGATCC
TTACCACAGTTTTTCTCATGGGAAATACATAATAAACCTTTCATCTTTTTTTTTTTCCTTTAAGAATTAA
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TCCTGTGTGTGAATTTAAAAAAAATACTTTACTTGGATATTCATGTAATATAAAAGGTTTGGTG
AAATGAACTTTAGTTAGGAAAAAGCTGGCATCAGCTTTCATCTGTGTAAGTTGACACCAATGTGTCATAA
TATTCTTTATTTTGGGAAATTAGTGTATTTTATAAAAAATTTAAAAAGAAAAAGACTACTACAGGTAA
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CTGGTATAAAGTTGGTTAAATTTTCATCTGTTAATAGATCATTAGGTAATATAATGTATGGGTTTCTAT
TGGTTTTTTGCAGACAGTAGAGGGAGATTTTGTAAACAAGGGCTTGTTACACAGTGATATGGTAATGATAA
AATTGCAATTTATCACTCCTTTTCATGTTAATAATTTGAGGACTGGATAAAAGGTTTCAAGATTAAATTT
TGATGTTCAAACCTTTGT

Figure 4: 5' cDNA fragment of human POSH (public gi:10432611; SEQ ID NO:4)

ctgagagacactgagcgagcgagcgcggtggggccgcacatctgcacagccgcccagccgctgccccg
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cagcagctgctccactgacttccccagcatcaccagtgcttctctggaggctgagccagtgccggat
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accaaaccagacaaggatagc

Figure 5: N terminus protein fragment of hPOSH (public gi:10432612; SEQ ID NO:5)

MDESALLDLLECPVCLERLDASAKVLPCQHTFCKRCLLGIVGSRNELRCPECRTLVGSGVEELPSNILLV
RLLDGIKQRPWKPGPGGGSGTNCNLRQSSTVANCSSKDLQSSQGGQQPRVQSWSPVVRGIPQLPCKAK
ALYNYEGKEPGDLKFSKGDIIILRRQVDENWYHGEVNGIHGFFPTNFVQIIKPLPQPPPQCKALYDFEVK
DKEADKDCLPFAKDDVLTVIRRVDENWAEGLADKIGIFPISYVEFNAAKQLIEWDKPPVPGVDAGECS
SAAAQSSTAPKHSDTKKNTKKRHSFTSLTMANKSSQASQNRHSMEISPPVLISSENPTAAARISELSGLS
CSAPSQVHISTTGLIVTPPPSSPVTTGPSFTFPDVPYQAALGTLPPLPPPPLLAATVLASTPPGATAA
AAAAGMGPRPMAGSTDQIAHLRPQTRPSVYVAIYPYTPRKEDELELRKGEMFLVFERCQDGWFKGTSMT
SKIGVFPGNVYAPVTRAVTNASQAKVPMSTAGQTSRGVTMVSPSTAGGPAQKLQGNVAGSPSVVPAVV
SAAHIQTSPQAKVLLHMTGQMTVNQARNAVRTVAHNQERPTAAVTPIQVQNAAGLSPASVGLSHHSLAS
PQPAPLMPGSATHTAAISISRASAPLACAAAAPLTSPSITSASLEAEPSGRIVTVLPGLPTSPDSASSAC
GNSSATKPKDKDS

Figure 6: 3' mRNA fragment of hPOSH (public gi:7959248; SEQ ID NO:6)

atttcatatgttgagtttaactcggctgctaagcagctgataagaatgggataagcctcctgtgccaggag
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cttggtatattcatgtaatatataaagggtttgggtgaaatgaacttttagttaggaaaaagctggcatcagct
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aatttttaaaaagaaaaagactactacaggttaagataattttttacctgtcttttctccatatttttaa
gctatgtgattgaagtacctctgttcatagtttcctgggtataaagttgggttaaaatttcatctgttaata
gatcattaggtaatataatgtatgggttttctattgggttttttgacagcagtagaggagattttgtaac
aagggtctgttacacagtgatgtggtaatgataaaattgcaatttatcactccttttcatgttaataatt
tgaggactggataaaagggtttcaagattaaaatttgatgttcaaacctttgt

Figure 7: C terminus protein fragment of hPOSH (public gi:7959249; SEQ ID NO:7)

ISYVEFNAAKQLIEWDKPPVPGVDAGECSSAAQSSSTAPKHSDDTKNTKKRHSFTSLTMANKSSQASQN
RHSMEISPPVLISSSNPTAAARISELSGLSCSAPSQVHISTTGLIVTPPPSSPVTTGPSFTFPDVPYQA
ALGTLNPPLEPPPLLAATVLAATPPGATAAAAAAGMPRPMAGSTDQIAHLRPQTRPSVYVAIYPYTPRK
EDELELRKGEMFLVFERCQDGFKGTSMTSKIGVFPNGYVAPVTRAVTNASQAKVPMSTAGQTSRGVTM
VSPSTAGGPAQKLQGNQVAGSPSVVPAAVVSAHIQTSPQAKVLLHMTGQMTVNQARNAVRTVAAHNQER
PTAAVTPIQVQNAAGLSPASVGLSHHSLASPQAPLMPGSATHTAAISISRASAPLACAAAAPLTSPTSIT
SASLEAEPSGRIVTVLPGLPTSPDSASSACGNSSATKPKDKSKKEKKGLLKL LSGASTKRKPRVSPASP
TLEVELGSAELPLQGAVGPELPPGGGCHGRAGSCPVDGDPVTTAVAGAALAQDAFHRKASSLDSAVPIAP
PPRQACSSLGPFVLESRPVVCERHRVVVSYPQSEAELELKEGDIVFVHKKREDGWFGKTLQRNGKTGLF
PGSFVENT

Figure 8: Human POSH full mRNA, Annotated Sequence (part 2)

TTGTGGACTTCCAGATGGTCAGGAGATGAGCAAAGGATTGGTATGTGACTCTGATGCCCCAGCACAGTTA
CCCCAGCGAGCAGAGTGAAGAAGATGTTTGTGTGGGTTTTGTAGTCTGGATTCGGATGTATAAGGTGTG
CCTTGTACTGTCTGATTTACTACACAGAGAACTTTTTTTTTTTTAAAGATATATGACTAAAATGGACA
ATTGTTTACAAGGCTTAACCTAATTTATTTGCTTTTTTAACTTGAACTTTTCGTATAATAGATACGTTCT
TTGGATTATGATTTTAAGAAATTTAATTTATGAAATGATAGGTAAGGAGAAGCTGGATTATCTCCTGT
TGAGAGCAAGAGATTTCGTTTTGACATAGAGTGAATGCATTTTCCCTCTCCTCCTCCCTGCTACCATAT
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AATGTATTTAGAAATATATTTAGTTTATAGCAGAAGCAGCTCAATTGTTTGGTTGGAAAGTAGGGGAAA
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TTAGTGAAGTGGTCTTTATGTCTCTCCAGCAGACAGACATCAAGGATGAGTTAACAGGAGACTACTC
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CTGGTATAAAGTTGGTTAAATTTTATCTGTAAATAGATCATTAGGTAATATAATGTATGGGTTTTCTAT
TGGTTTTTTGCGACAGTAGAGGGAGATTTGTAAACAGGGCTTGTACACAGTGATATGGTAATGATAA
AATTGCAATTTATCACTCCTTTTCATGTAAATAATTGAGGACTGGATAAAAGGTTTCAAGATTAAAAAT
TGATGTTCAAACCTTTGT

Figure 9: Domain Analysis of Human POSH

Domain Name	begin	end	E-value
<u>RING</u>	12	52	1.06e-08
<u>SH3</u>	137	192	2.76e-19
<u>SH3</u>	199	258	4.84e-15
<u>low complexity</u>	366	384	-
<u>low complexity</u>	390	434	-
<u>SH3</u>	448	505	2.40e-19
<u>low complexity</u>	547	563	-
<u>low complexity</u>	652	668	-
<u>low complexity</u>	705	729	-
<u>SH3</u>	832	888	1.47e-14

Figure 10: Diagram of Human POSH Nucleic Acids

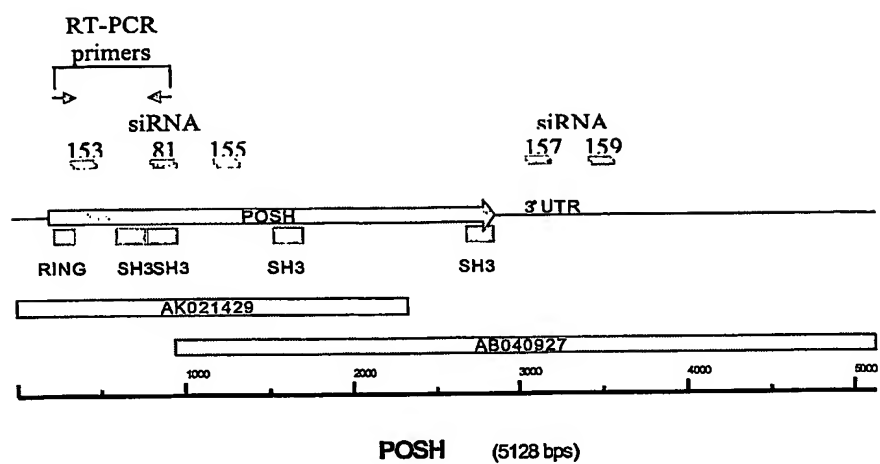


Figure 11: Reduction in Full Length POSH mRNA by siRNA Duplexes

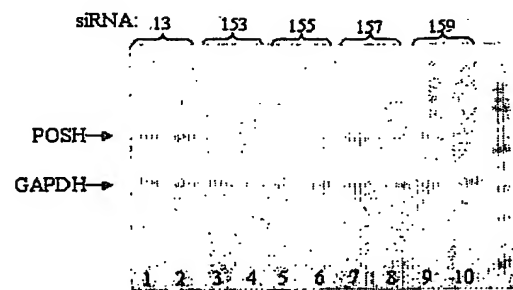


Figure 12: POSH Affects Release of VLP from Cells

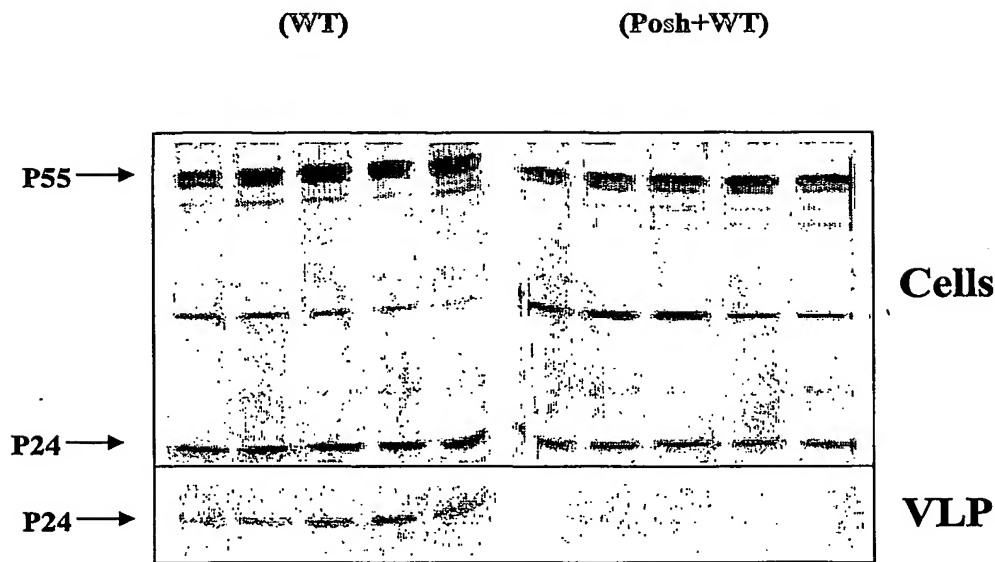


Figure 13: Release of VLP from Cells at Steady State

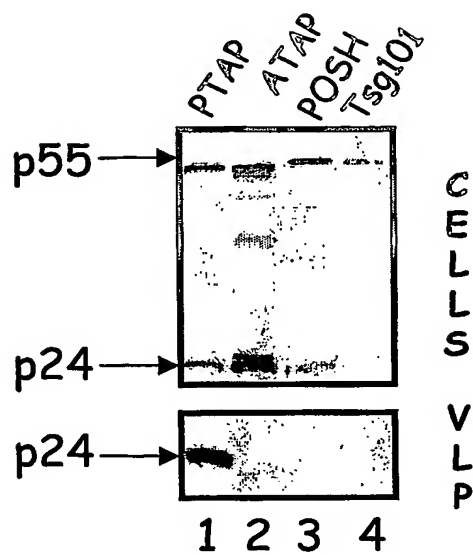


Figure 14: Mouse POSH mRNA sequence (public gi:10946921; SEQ ID NO: 8)

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AAGCCGTTTTCTCACTAAAGTCACTCAAGATGGATGAGTCTGCCTTGTGGACCTTCTGGAGTGCCCTGT
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GTTACAGCAAAGGCGACACCATCATTCTGCGCGGACAGGTGGATGAGAATTGGTACCAACGGGAAGTCAGC
GGGGTCCACGGCTTTTCCCACTAATCTCGTGCAGATCATCAAACCTTTACCTCAGCCCCCGCTCAGT
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CGACGTACTGACCGTGATCCGAGAGTGGATGAAAACCTGGGCTGAAGGAATGCTGGCAGATAAAATAGGA
ATATTTCCAATTTTATACGTGGAGTTTAACTCAGCTGCCAAGCAGCTGATAGAGTGGGATAAGCCTCCCG
TGCCAGGAGTGGACACGGCAGAATGCCCTCAGCGACGGCGCAGAGCACCTCTGCCTCAAAGCACCCCGA
CACCAAGAGAACACCGAGAACGACACTCCTTCACTCCCTCACCATGGCCAAAGTCTTCCCAAGGG
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TGTTTGAGCGTTGCCAGGACGGCTGGTACAAAGGGACATCGATGCATACAGCAAGATAGCGCTTTCC
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GCTTCTCTGGCCTCCCCAAATATGACCAAGTGCATGTTGGAGACAGAGCCAGTGGTGCACAGTGACCA
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AGACAAGGACAGTAAGAAAGAAAAAAGGGCCTACTGAAGCTGCTTTCTGGTGCCTCCACCAAACGCAAG
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GGCCTGTGTTTGTGAAGGCAAGGGTGGTGGTTTCTTACCCTCCTCAGAGTGAGGCCGAACCTGAACT
CAAGGAAGGAGATATTGTGTTTGTTCATAAGAAACGAGAGGACGGCTGGTTCAAAGGCAGTTACAGAGG
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TCTAATGGACTTTACAGATAAATGTCTTTTTTTTTTAAAGATGTATACTAAATGGACAATGTTTTACA
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Figure 15: Mouse POSH Protein sequence (Public gi: 10946922; SEQ ID NO: 9)

MDESALLDLLECPVCLERLDASAKVLPCQHTFCKRCLLGIVGSRNELRCPECRTLVGSGVDELPSNILLV
RLLDGIKQRPWKPGPGGGGGTTCTNTLRAQGSTVVNCGSKDLQSSQCGQPRVQAWSPPVRGIPQLPCKA
ALYNYEGKEPGDLKFSKGDITILRRQVDENWYHGEVSGVHGFFPTNFVQIIKPLPQPPPPQCKALYDFEVK
DKEADKDCLPFAKDDVLTVIRRVNENWAEGLADKIGIPPISYVEFNAAKQLIEWDKPPVPGVDTAEC
SATAQSTSASKHPDTKKNTRKRHSFTSLTMANKSSQGSQNRHSMEISPPVLISSSNPTAAARISEL
SGLS
CSAPSQVHISTTGLIVTPPPSSPVTTGPAFTFPSPDVPYQAALGSMNPPLPPPPPLAATVLASTP
SGATAA
VAAAAAAAAAAGMGRPVMGSSEQIAHLRPQTRPSVYVAIYPYTPRKEDELELRKGMFLVFERCQD
GWY
KGTSMHTSKIGVFPNGYVAPVTRAVTNASQAKVSMSTAGQASRGVTMVSPSTAGGPTQKPQNGV
AGNPS
VVPTAVVSAAHITSPQAKVLLHMSGQMTVNQARNAVRTVAHNSQERPTAAVTPIQVQNAACLG
PASVGL
PHHSLASQPLPPMAGPAAHGAASVTSRTNAPMACAAGASLASPNMTSAMLETEPSGRRTVTIL
PGLPTS
PE
SAASACNGSSAGKPKDKSKKEKKGLLKLKSGASTKRKPRVSPPASPTLDVELGAGEAPLQGA
VGP
PELPLG
GSHGRVGCPTDGDGPVAAAGTAALAQDAFHRKTSSLDASVPIAPPPRQACSSLGPMNEARPV
VCERHRV
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Figure 16: *Drosophila melanogaster* POSH mRNA sequence (public gi:17737480; SEQ ID NO:10)

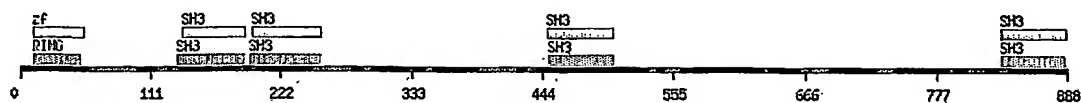
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TGAAACACAGCCGGAAGGGCCAAACCTCAGCCGCCAGCGGAATCAGTGGCCCCGCTGACAAACCACTA
CTCCAGCTGCAGTCACATCAGCAATCTCATCAGCCGGCTCGTCAAGCAACGTCGATTTCTACTCCCCC
ACGCCATATGCCCTCTTGACTTCGCCTCCGGTGAAGCCACCGATCTAAAGTTCAAGAAAGGGGATCTGAT
ACTGATCAAGCATCGCATCGACAAACAATGGTTTGTGGGTCAAGCGAATGGTCAGGAGGGGATCTGCC
ATCAACTACGTCAAGGTATCGGTTCCGCTGCCCATGCCGAGTGCAATGCCATGTATGACTTTAAGATGG
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Figure 17: *Drosophila melanogaster* POSH protein sequence (public gi:17737481; SEQ ID NO:11)

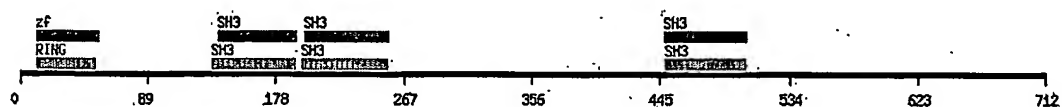
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Figure 18: POSH Domain Analysis

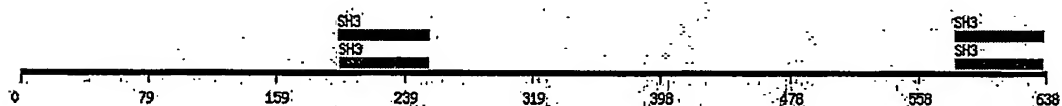
hPOSH protein sequence :



N terminus protein fragment of hPOSH (public gi:10432612):



C terminus protein fragment of hPOSH (public gi:7959249):



Mouse POSH Protein sequence (Public gi: 10946922):



Drosophila melanogaster POSH protein sequence (public gi:17737481)



Figure 19: Human POSH has ubiquitin ligase activity

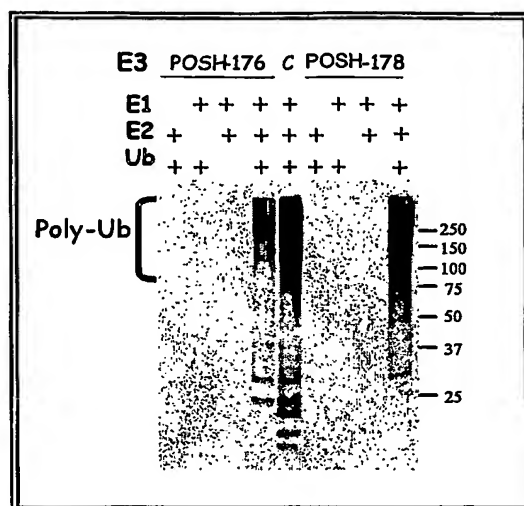


Figure 20. PLD activity in medium of transfected cells

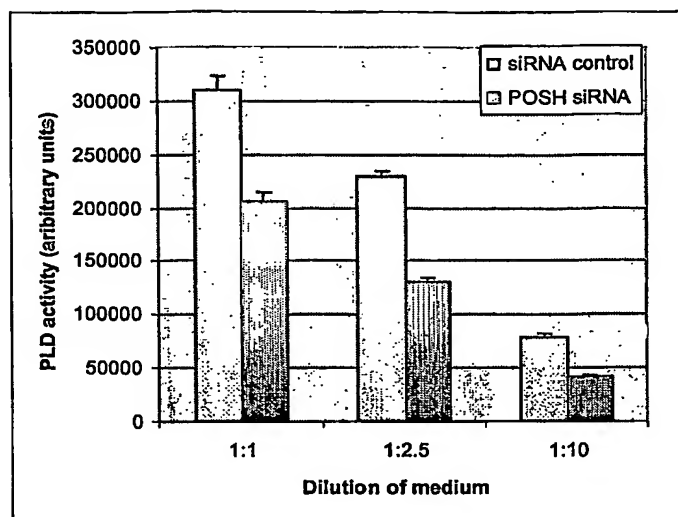
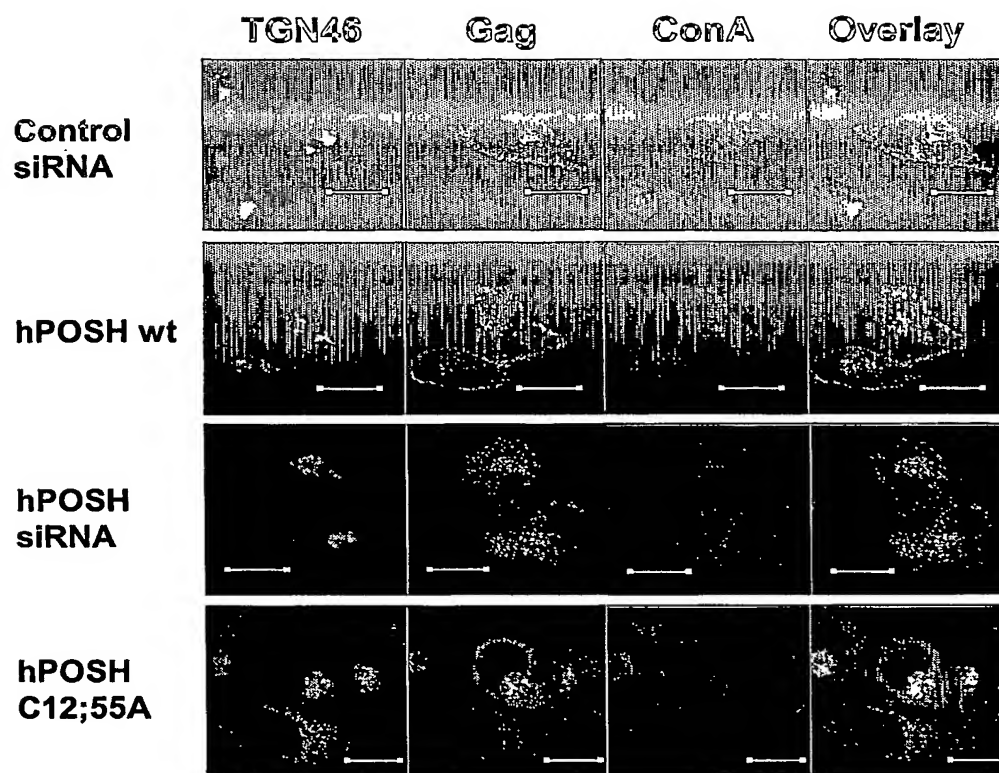
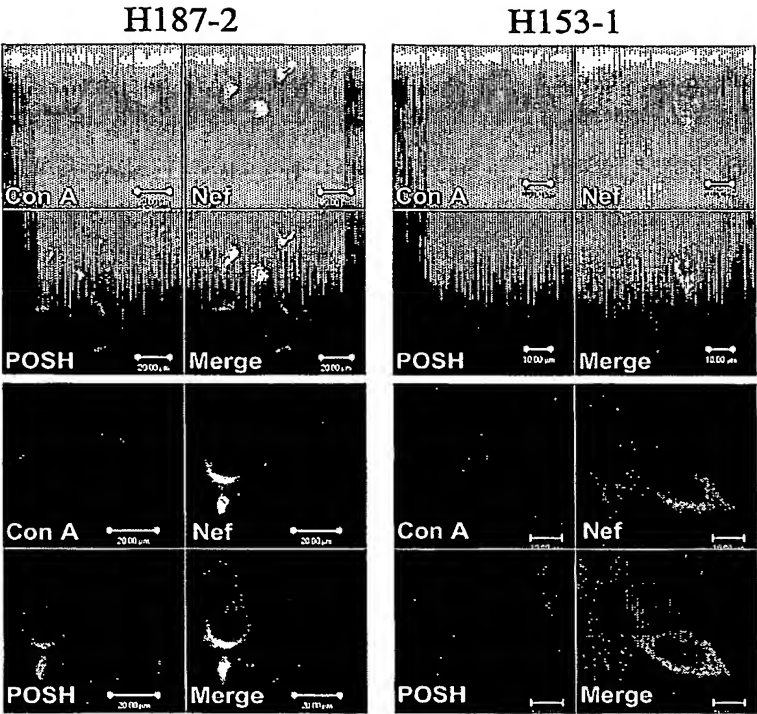


Figure 21.



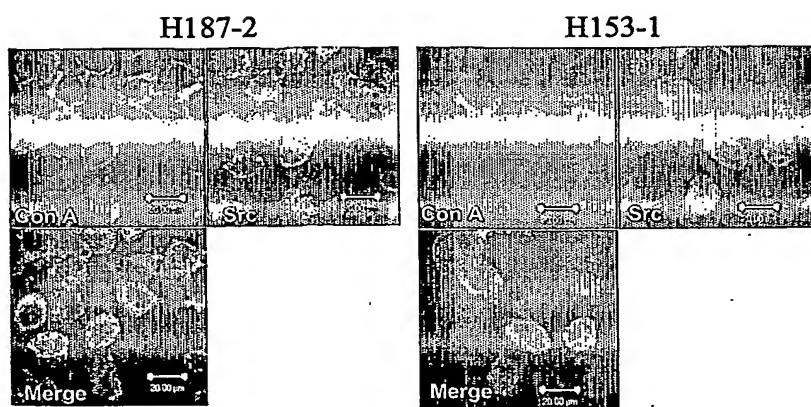
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Figure 22.



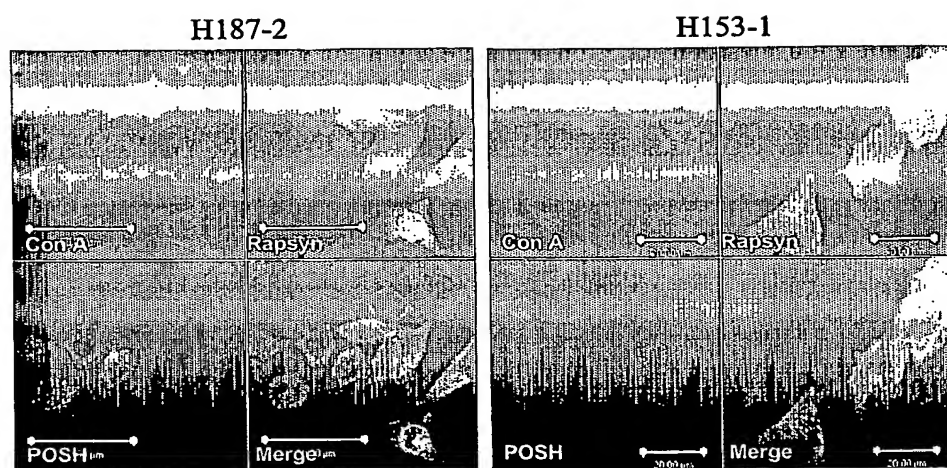
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Figure 23.



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Figure 24.



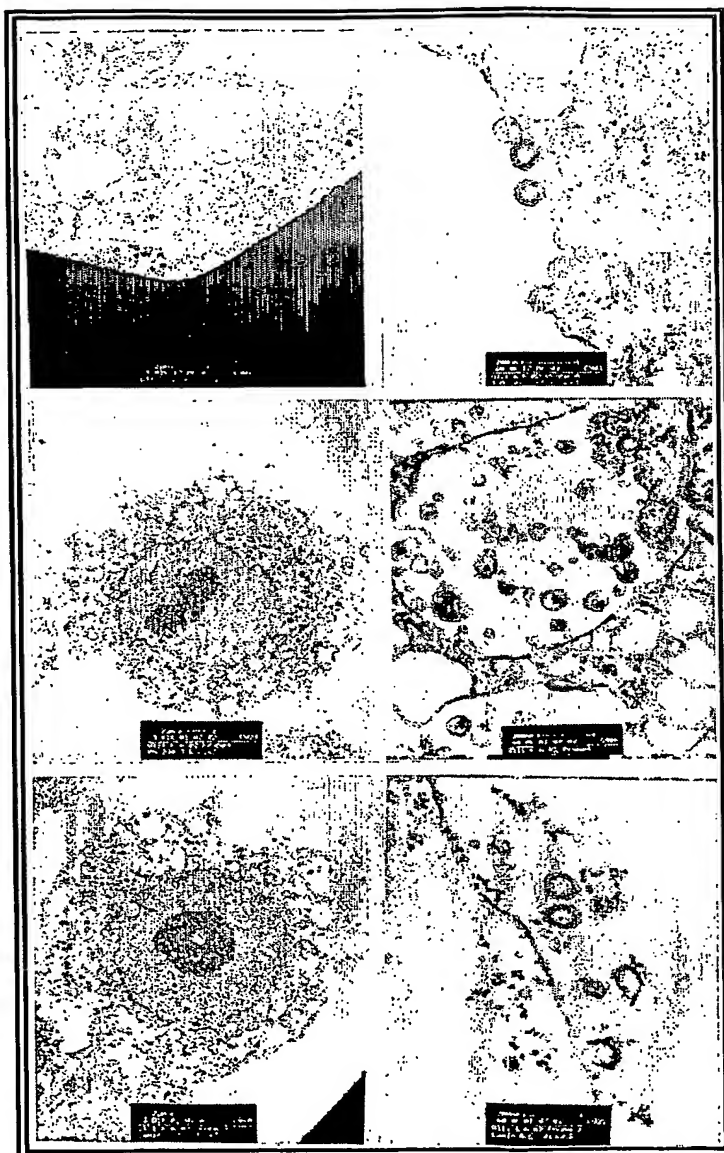
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Figure 25.

SiRNA-Tsg101

SiRNA-POSH

Control



BEST AVAILABLE COPY

Figure 26.

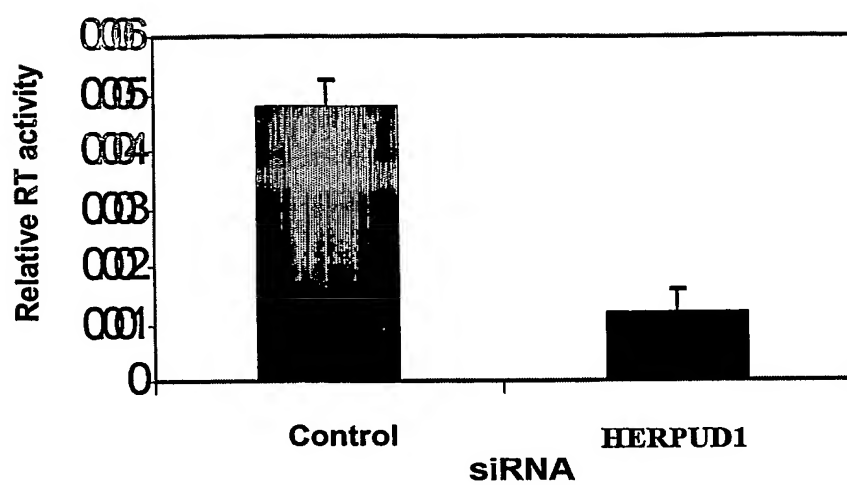
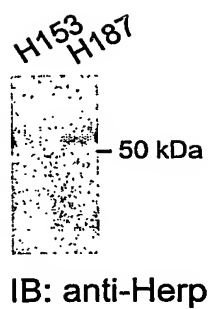


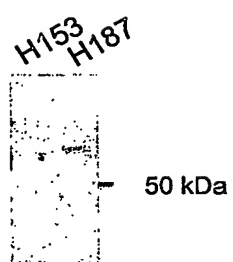
Figure 27.

A



IB: anti-Herp

B



IP: anti-Flag (Ubi)
IB: anti-Herp

Figure 28.

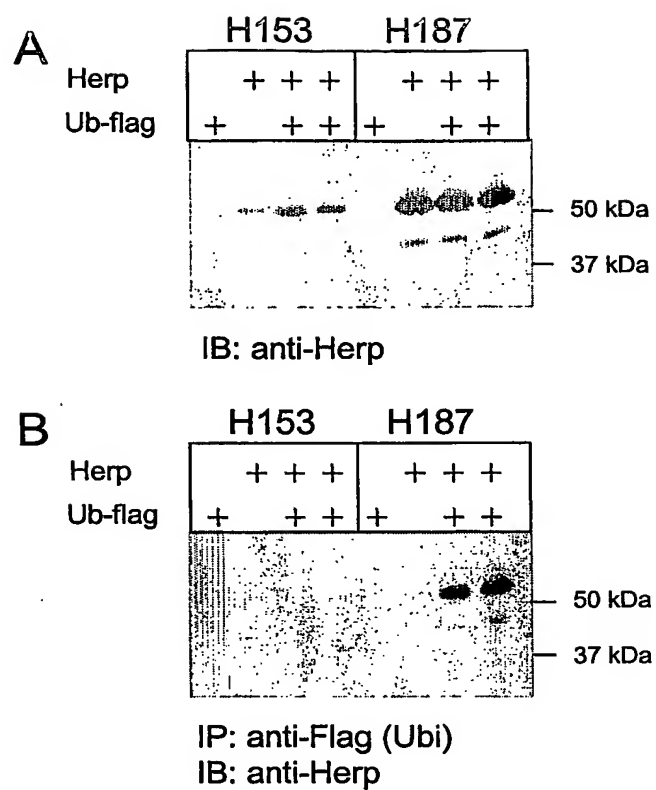


Figure 29.

